Clinical, Experimental, and Genomic Differences between Intermediately Pathogenic, Highly Pathogenic, and Epidemic *Streptococcus suis*

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(See the editorial commentary by Segura, on pages 4–6.)

Background. *Streptococcus suis* emerged to cause an unusual outbreak of streptococcal toxic-shock-like syndrome (STSLS) in 2005. The mechanisms involved are unknown.

Methods. Clinical, laboratory, and epidemiologic data on patients infected with culture-confirmed *S. suis* were analyzed. The strain involved in the outbreak, "epidemic" strain ST7, was compared with both a classical highly pathogenic strain, ST1, and an intermediately pathogenic strain, ST25, to determine both its capacity to induce cytokines in experimentally infected mice and its genomic difference.

Results. Of 38 patients infected with culture-confirmed *S. suis*, 14 presented with STSLS. During the early phase of the disease, serum levels of interleukin (IL)–1β, IL-6, IL-8, IL-12p70, interferon-γ, and tumor necrosis factor–α were more elevated in patients with STSLS than in those with meningitis only. Serum levels of proinflammatory cytokines were significantly higher in mice infected with ST7 than in those infected with either ST1 or ST25. Genomic comparisons with ST25 showed that ST1 had acquired 132 genomic islands, including 5 pathogenicity islands, and that ST7, the epidemic strain, had acquired an additional 5 genomic islands.

Conclusion. Intermediately pathogenic strain ST25 has evolved to become highly pathogenic strain ST1, which, in turn, has more recently evolved to become epidemic strain ST7. ST7 has the ability to stimulate the production of massive amounts of proinflammatory cytokines, leading to STSLS.

Streptococcus suis has been described as one of the most important and prevalent pathogens in swine [1]. It is also considered an important zoonotic agent [1]. At present, there are 35 recognized serotypes of *S. suis*, of which serotype 2 is considered to be the most prevalent and virulent in pigs and humans with *S. suis*–related disease [1]. Putative virulence markers—such as muramidase-released protein (MRP), extracellular factor (EF), and hemolysin (suilysin)—have been described [2]. Interestingly, strains from North America are negative for these 3 factors and are considered to be of lower virulence [2].

Since 1968, when the first human case was reported, 250 human cases had been identified worldwide as of June 2005. The majority of cases have resulted in meningitis, often in association with hearing loss [1]. During July and August of 2005, however, a sudden outbreak of 215 human cases occurred in Sichuan, China [3]. Of 215 patients, 61 (28%) presented with an unusual streptococcal toxic-shock–like syndrome (STSLS), with high (62%) mortality in previously healthy farmers [3]. Both the clinical presentation and the epidemiology of the Sichuan outbreak had not been observed previously [4, 5], provoking the scientific community to have a strong interest in the possible involvement of superantigens [6].

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Multilocus sequence typing, the method of choice for addressing questions related to genotyping in epidemiology, population, and evolutionary-biology studies, has identified 94 sequence types (STs) of *S. suis*. Of these types, ST7 has been recognized as an "epidemic" strain and the causative agent of the Sichuan outbreak [7, 8]. It also has been found that strains isolated from human cases that had occurred worldwide up to 2005 could be grouped into an ST1 complex (including strains ST1, ST6, ST7, and ST84) and an ST29 complex (including strains ST29 and ST25) [8]. The *S. suis* ST1 complex included most of the serotype 2 isolates that previously had been recovered from infected humans in Europe, whereas *S. suis* ST25 was found to include North American strains [7]. A phylogenetic tree of *S. suis*, constructed by use of the concatenated sequences from 7 housekeeping genes used in multilocus-sequence-typing analysis, showed that ST25 is positioned at an early stage of evolution and that ST7 is derived from ST1 via a single nucleotide change in housekeeping gene *thyA*.

Thus far, *S. suis* ST7 has been isolated only in China, where its virulence is assumed to have increased, as demonstrated by the fact that its toxicity to peripheral blood mononuclear cells (PBMCs) is greater than that of ST1 [8]. The objective of the present study was to test the hypothesis that *S. suis* epidemic ST7 recently evolved from a highly pathogenic clone prevalent in other parts of the world. ST7 has the ability to cause severe shock; since the first human case was reported in Hong Kong in 1996, ST7 has emerged to cause a small outbreak in Jiangsu in 1998 and then has spread to cause the largest reported outbreak of severe shock in 2005 [7, 8].

MATERIALS AND METHOD

Clinical analysis and characterization of field strains. The present study included only patients for whom both cultures of *S. suis* from a sterile site (blood and/or cerebrospinal fluid) and medical records were available. Cultures were obtained and identified as described elsewhere [9, 10]. The presence of virulence genes was confirmed by polymerase chain reaction and sequencing [11, 12]. Pulse-field gel electrophoresis of the isolates digested with restriction enzyme *Sma*I was performed as described elsewhere [8].

Data on the patients were extracted from medical charts by a team of trained microbiologists and clinicians. After patients gave verbal informed consent, serum was obtained and immediately was frozen at −70°C, for cytokine assays performed at the central laboratory in Beijing. STSLS caused by *S. suis* was diagnosed if the patient presented with a high fever, hypotension, and 2 or more of the following conditions: renal impairment, liver abnormalities, acute respiratory distress, coagulopathy, and extensive tissue necrosis.

To elucidate the inflammatory response during the early phase of infection, serum samples were collected from patients ⩽3 days after admission and were diluted sequentially, from 4-fold to 64-fold, for analysis. Cytokines—including interleukin (IL)–6, IL-8, IL-12, interferon (IFN)–γ, IL1-β, and tumor-necrosis factor (TNF)–α—were assayed by use of Fluorokine MAP Multiplex Human Cytokine Panel A of the Luminex 100 Analyzer with the X-Y Platform (Luminex). Cytokine levels were analyzed by use of Flowmetrix software (Miraibio Masterplex QT2.0; Luminex) [13].

Experimental strains. Strains of *S. suis* were divided into 3 groups—namely, an intermediately pathogenic group represented by strain ST25 89/1591, a highly pathogenic group represented by strain ST1 GZ1, and an "epidemic" group represented by strain ST7 SC84—that were based on published clinical, epidemiological, pathogenic, and phylogenetic data. Intermediately pathogenic strain ST25 89/1591 is a typical North American strain that is highly prevalent in Canada and United States [1]. Although ST25 has been demonstrated to be relatively virulent in pigs [14], it does not possess the virulence markers MRP, EF, and suilysin and seems to be less virulent than strains possessing these markers' European counterparts [1, 15]. It is notable that, thus far, none of the cases of toxic shock-like syndrome, septic shock, or even death that have been attributed to ST25 have been reported in North America. Highly pathogenic strain ST1 GZ1, isolated in 2005 from a patient in China who had septicemia, is representative of most strains isolated from humans in Europe and Asia before the 2005 Sichuan outbreak. ST1 has been associated with a few cases of septic shock and death in some countries in Europe and Asia. In the present study, ST7 SC84 is considered to be an epidemic strain because it was responsible for the large outbreak of STSLS in 2005, as mentioned above.

Experimental virulence of* S. suis *strains. The virulence of *S. suis* strains was evaluated by use of an experimental mouse-infection model and a cytotoxicity assay with PBMCs freshly isolated from healthy donors [4, 8]. Five 6-week-old C57BL/6 mice per group were injected ip with 1×10^6 cfu of one of the representative strains in 1 mL of Todd-Hewitt broth. At 8 h after injection, mice were killed, and peripheral blood was collected from them. All serum samples were diluted 4-fold to 10-fold, and cytokines were analyzed by use of the Luminex 100 Analyzer, as described above. To determine bacterial cytotoxicity, PBMCs from healthy donors were seeded, at a final concentration of 10^6 cells/mL, in 24-well plates and were incubated, for 4 h at 37°C, in the presence of *S. suis* epidemic strain ST7 SC84, highly pathogenic strain ST1 GZ1, or intermediately pathogenic strain ST25 89-1591, at a concentration of 10^7 cfu/mL, as described elsewhere [8]. Bacterial cytotoxicity was evaluated on the basis of measurement of lactate dehydrogenase, which was determined by use of a Cyto-Tox 96 Cytoxicity Kit (Promega) according to the manufacturer's instructions. The percentage of cytotoxicity was calculated as follows: (sample optical density [OD] at 490 nm [OD_{490}] − OD0%)/(OD100% − OD0%) ×

100, where OD0% represents the OD_{490} of uninfected cells and OD100% represents the OD_{490} of cells treated, as recommended by the manufacturer, with lysis buffer. Wells with cell-culture medium alone served as controls.

Genome sequencing and comparative genomic analysis. The genome of *S. suis* highly pathogenic strain ST1 GZ1 was sequenced by a whole-genome shotgun method [16]. Approximately 8.9-fold genome coverage was achieved by generating 25,014 sequences, with an average length of 1.5 kb, from a small-insert plasmid library. Sequences were assembled by use of Phrap [17], Phred, and Consed software. Coding regions were identified by use of the software-analysis package EMBOSS. All putative open reading frames were searched by use of the NCBI (National Center for Biotechnology Information), Swissport, and KEGG (Kyoto Encyclopedia of Genes and Genomes) nonredundant-nucleotide and nonredundant-protein databases. BLASTN (Basic Local Alignment Search Tool, Nucleotides) and ART (Artemis Comparison Tool) were used for pairwise alignment of the genomes.

Statistical analysis. Fisher's exact test, Student's *t* test, or the Kruskal-Wallis test were used for statistical analyses.

RESULTS

***Clinical presentation and serum levels of cytokine in patients infected with culture-confirmed* S. suis.** Cultures were performed for 174 of 215 cases reported between 15 July and 11 August 2005; at 8 tertiary hospitals in Sichuan, China, a total of 84 blood or cerebrospinal-fluid cultures were positive for *S. suis*, and medical records were obtained for 38 of the 84 patients infected with culture-confirmed *S. suis*. All isolates were positive for the putative virulence factors MRP, EF, and suilysin, as determined by PCR. Pulse-field gel electrophoresis of the isolates digested with restriction enzyme *Sma*I showed that the 38 isolates were genetically identical [8] (results not shown).

The patients' clinical features at presentation are listed in table 1. Of the 38 patients infected with culture-confirmed *S. suis* for whom medical records were available, 14 (37%) had STSLS, and these 14 patients were more likely to have skin manifestations, such as petechiae, peripheral cyanosis, thrombocytopenia, and abnormal coagulation profiles that are characteristic of purpura fulminans (table 1). Also, mortality was high among these 14 patients—9 (64%) of them died—whereas no deaths were reported among the 24 patients with meningitis only. It is to be noted that the median time from disease onset to death was 23 h in these 9 patients; the time from disease onset to death was >3 days (i.e., 84 h and 96 h) for only 2 of the 9 patients and was much shorter for the other 7: 4 patients died <20 h (range, 13–18 h) after disease onset, and 1 patient each died 23, 26, and 28 h after disease onset.

At admission, serum samples were obtained from 15 consenting patients, 6 of whom had STSLS and 9 of whom had meningitis only. Serum levels of all 6 cytokines measured—specifically, IL-1β, IL-6, IL-8, IL-12p70, IFN-γ, and TNF-α—were significantly higher in the patients with STSLS than in the patients with meningitis only ($P < .05$) (table 1).

***Cytotoxicity to PBMCs, and serum levels of cytokine, in mice infected with representative intermediately pathogenic, highly pathogenic, or epidemic strains of* S. suis.** After 4 h of incubation, the 3 *S. suis* strains induced significantly different levels of cytotoxicity to PBMCs, as shown in figure 1. Highly pathogenic strain ST1 GZ1 was more toxic to PBMCs than was intermediately pathogenic strain ST25 89/1591, and epidemic strain ST7 SC84 induced the highest level of cytotoxicity to PBMCs and was significantly more toxic than was ST1 GZ1. Figure 1 also shows distinct profiles of serum levels of cytokines in mice 8 h after ip infection with different strains of *S. suis*. Production of TNF-α, IL-6, keratinocyte chemoattractant (KC), monocyte chemoattractant protein–1 (MCP-1), IL-12p70, and IL-1β, was significantly higher ($P = .001$, .002, .002, .013, .01, and .001, respectively) in mice infected with ST7 SC84 than in mice infected with ST1 GZ1. Production of TNF-α, IL-6, KC, MCP-1, IL-12p70, and IL-1β, was significantly higher ($P = .003$, .016, .009, .003, .003, and .001, respectively) in mice infected with ST1 GZ1 than in mice infected with ST25 89/1591. Last, serum levels of IFN-γ were significantly higher ($P < .001$ in both cases) in mice infected with either ST1 GZ1 or ST7 SC84 than in mice infected with ST25 89/1591 (figure 1). However, serum levels of IFN-γ were not significantly different ($P = .162$) between ST1 GZ1 and ST7 SC84.

***Genomic islands lost and acquired by highly pathogenic strain ST1 and epidemic strain ST7 of* S. suis.** The genome of highly pathogenic strain ST1 GZ1 is a single circular chromosome of 2,038,034 bp with a G+C content of 41.44% (CP000837). No plasmids were found. The chromosome has 1987 predicted coding sequences (CDSs); their average length is 909 bp, and the longest is 7664 bp (figure 2). The genome sequence of ST1 GZ1 and the draft sequence of intermediately pathogenic strain ST25 89/1591 (http://genome.jgi-psf.org/draft_microbes/strsu/strsu.home.html) share a common backbone sequence that is colinear. The homology is punctuated by deletions and insertions of hundreds of genes, which have been designated as "lost islands" (LIs) or "acquired islands" (AIs). LIs were found to be present in ST25 85/1591 but not in either ST1 GZ1 or ST7 05ZYH33, whereas AIs are present in ST1 GZ1 and ST7 05ZYH33 but not in ST25 85/1591 [18]. The AIs and LIs were also compared with ST25 89/1591's individual contiguous sequences that could not be linked to others; the results showed that ST1 GZ1 has 64 LIs and that ST7 05ZYH33 has 54 LIs (table 2; also see tables A1 and A2 in the appendix, which is available only in the electronic edition of the *Journal*).

Comparative analyses revealed that strain ST1 GZ1 gained 132 AIs, including 5 pathogenicity islands containing genes encoding virulence factors such as suilysin, extracellular protein factor, superoxide dismutase A, extracellular serine protease,

Table 1. Clinical features of patients infected with *Streptococcus suis* and presenting with streptococcal toxic-shock-like syndrome (STSLS) or with meningitis only.

	Patients with STSLS	Patients with meningitis only	*P*
Clinical presentations	*n* = 14	*n* = 24	
Temperature, mean ± SD, °C	38.5 ± 1.4 (*n* = 13)	38.2 ± 1.2 (*n* = 24)	.57
History of fever with chills, no. of patients	10	15	.73
Headache, no. of patients	4	19	.005
Malaise, no. of patients	9	14	>.999
Myalgia, no. of patients	5	9	>.999
Arthralgia, no. of patients	2	4	>.999
Peripheral cyanosis, no. of patients	10	4	.001
Petechiae, no. of patients	8	4	.014
Nausea, no. of patients	7	11	>.999
Vomiting, no. of patients	6	8	.73
Abdominal pain, no. of patients	3	3	.65
Diarrhea, no. of patients	6	1	.006
Altered sensorium, no. of patients	8	6	.081
Neck rigidity, no. of patients	5	17	.047
Hearing loss, no. of patients	3	6	>.999
Dyspnea, no. of patients	6	4	.13
Cough, no. of patients	3	2	.34
Laboratory-examination results	*n* = 14	*n* = 24	
White-blood-cell count, mean ± SD, cells/L	12.9 ± 6.9 (*n* = 14)	13.8 ± 5.0 (*n* = 23)	.68
Hemoglobin, mean ± SD, g/L	140.4 ± 22.1 (*n* = 14)	137.3 ± 22.5 (*n* = 22)	.69
Platelet count, mean ± SD, cells/L	78.9 ± 29.1 (*n* = 14)	129.9 ± 70.0 (*n* = 22)	.005
Blood-urea nitrogen, mean ± SD, mmol/L	11.6 ± 5.3 (*n* = 13)	6.7 ± 2.1 (*n* = 18)	.007
Creatinine, mean ± SD, μmol/L	174.0 ± 101.3 (*n* = 13)	73.6 ± 15.5 (*n* = 22)	.004
Total bilirubin,[a] mean, μmol/L	20.9 (*n* = 12)	17.4 (*n* = 21)	.125
Serum albumin, mean ± SD, g/L	33.3 ± 5.6 (*n* = 12)	36.2 ± 4.7 (*n* = 21)	.11
Alanine aminotransferase,[a] mean, IU/L	106 (*n* = 12)	34 (*n* = 21)	.015
Aspartate aminotransaminase, mean ± SD, IU/L	399.4 ± 332.3 (*n* = 10)	43.4 ± 19.2 (*n* = 21)	<.001
Prothrombin time,[a] mean, s	16.4 (*n* = 13)	13.0 (*n* = 14)	.014
Activated-partial-thromboplastin time, mean ± SD, h	55.7 ± 28.9 (*n* = 11)	28.2 ± 5.4 (*n* = 14)	.011
Creatine kinase,[a] mean, μmol/L	323.50 (*n* = 6)	124.50 (*n* = 12)	.019
Lactate dehydrogenase, mean ± SD, IU/L	657.0 ± 489.1 (*n* = 6)	206.5 ± 81.7 (*n* = 13)	.074
Serum levels of cytokines, median (interquartile range), pg/mL	*n* = 6	*n* = 9	
Interferon-γ	282 (3.4–1200)	2[b] (2–271)	<.001
Tumor necrosis factor–α	228 (4.4–450)	18 (2.4–210)	.004
Interleukin-6	191,816 (202–232,258)	391 (36–8774)	.003
Interleukin-8	63,462 (1029–178,698)	1248 (91–10,162)	.020
Interleukin-12p70	550 (77–3068)	52 (26–786)	.012
Interleukin-1β	87 (12–1065)	8 (1–366)	.048

[a] Includes previous leptospirosis [1], psychiatric illness [1], chronic hepatitis B [1], peptic ulcer disease [2], gastritis [1], hypertension [1], and hyperthyroidism [1].

[b] In 8 of 9 serum samples from patients with meningitis only, the interferon-γ level was <2 pg/mL, the lowest value detectable by the kit used.

and prolipoprotein signal peptidase [2, 19, 20]; other AIs included 1 conferring antibiotic resistance, 5 encoding determinants of cell structure, 11 for DNA/RNA processing, 9 for regulation, 6 for DNA recombination, 6 for signal transduction, 43 for metabolism, 22 for transport, 1 for immunogenic protein, and 21 of unknown function. The total size of the AIs is 478,262 bp. Putative virulence genes present in strain ST25 89/1591 included those encoding 2-glyceraldehyde-3-phosphate dehydrogenase, sortases, nicotinamide adenine dinucleotide phosphate–dependent glutamate dehydrogenase, heat-shock protein Hsp70, arginine deiminase, hyaluronidase, fibrinogen binding protein, serum opacity factor, and elongation factor Ts (table 2 and figure 2;

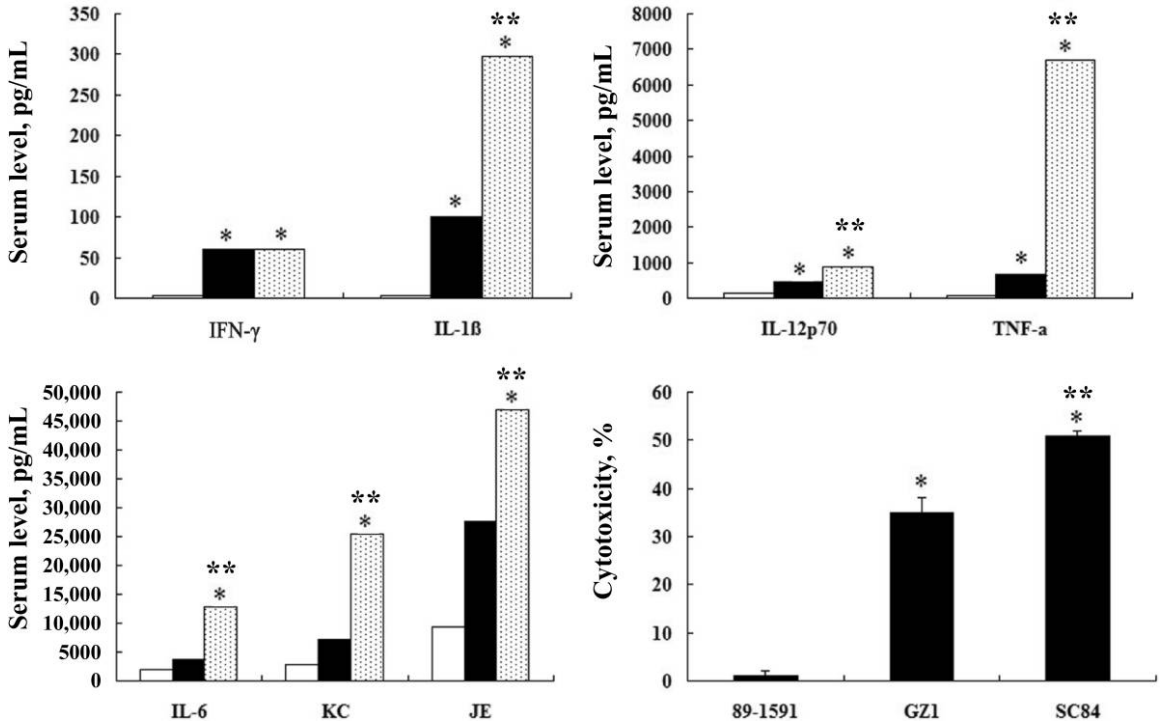

Figure 1. Virulence of intermediately pathogenic strain ST25 89/1591 (*white bars*), highly pathogenic strain ST1 GZ1 (*black bars*), and epidemic strain ST7 SC84 (*speckled bars*) of *Streptococcus suis*. Serum levels of cytokines in C57BL/6 mice 8 h after infection with 10^6 live *S. suis* are presented in the left-hand panels. The lower right-hand panel shows the cytotoxicity to PBMCs that 10^7 live *S. suis* have at 4 h incubation, presented as the percentage of released lactate dehydrogenase. All data are from 3 independent experiments. $^*P < .05$, for comparison of the group value versus the value for ST25 89/1591 alone; $^{**}P < .05$ for comparison of the group value versus the value for ST1 GZ1 alone.

also see table A2 in the appendix, which is available only in the electronic edition of the *Journal*) [1].

On the basis of comparisons with the available genome of another epidemic ST7 strain (05ZYH33), a total of 5 AIs with 64 CDSs comprising 66,829 bp were found to be unique to strain ST7 SC84; these AIs were classified as ST7-specific genomic islands and include 1 encoding determinants for tetracycline resistance and the NisK-NisR–like 2-component signal-transduction system, 1 encoding determinants for the SalK-SalR–like 2-component signal-transduction system, 1 encoding a recombinase, 1 encoding an adenosine triphosphate–binding cassette (ABC)–type metal-ion transporter, and 1 encoding a peptide ABC transporter (figure 3) [16]. Factors involved in the NisK-NisR-like and SalR-SalK–like 2-component signal-transduction systems regulate the biosynthesis and immunity of nisin and salivaricin, respectively [21, 22], and the latter system is also involved in intraspecies and interspecies signaling between *S. salivarius* and group A streptococcus (GAS) [21]; however, neither nisin-like protein nor salivaricin-like protein could be identified.

DISCUSSION

The outbreak of *S. suis* infection in Sichuan, China, during 2005 showed definitive features of STSLS, including sudden onset of high fever, hypotension, and multiple-organ dysfunction, such as acute respiratory distress syndrome, liver and heart failure, disseminated intravascular coagulation, and acute renal failure [3, 23]. By contrast, streptococcal toxic-shock syndrome (STSS) is toxin mediated and is associated primarily with superantigens. No putative superantigen or homologous gene was identified in the genomes of *S. suis* isolates associated with the Sichuan outbreak, indicating that a unique mechanism was involved [16].

To elucidate possible mechanisms underlying the unusually virulent outbreak of *S. suis*, we analyzed clinical and epidemiological data on patients infected with culture-confirmed *S. suis* who presented with STSLS. There are also important differences, in epidemiology and clinical presentation, between STSS due to GAS and STSLS in the patients infected with culture-confirmed *S. suis* [3, 23, 24]. For example, abrupt severe pain and soft-tissue infection and rash, which are common in STSS, were rare in the cohort that we studied, whereas vomiting and diarrhea, which are uncommon in STSS due to GAS, were frequently observed. The results of the present study suggest that *S. suis*–related disease in the Sichuan outbreak was much more severe than previously had been reported [3]: in the cohort that we studied, both the percentage of patients presenting with STSLS and mortality were higher than previously had been reported [3]. Patients were more likely to have skin manifestations such as petechiae, as well as peripheral cyanosis, thrombo-

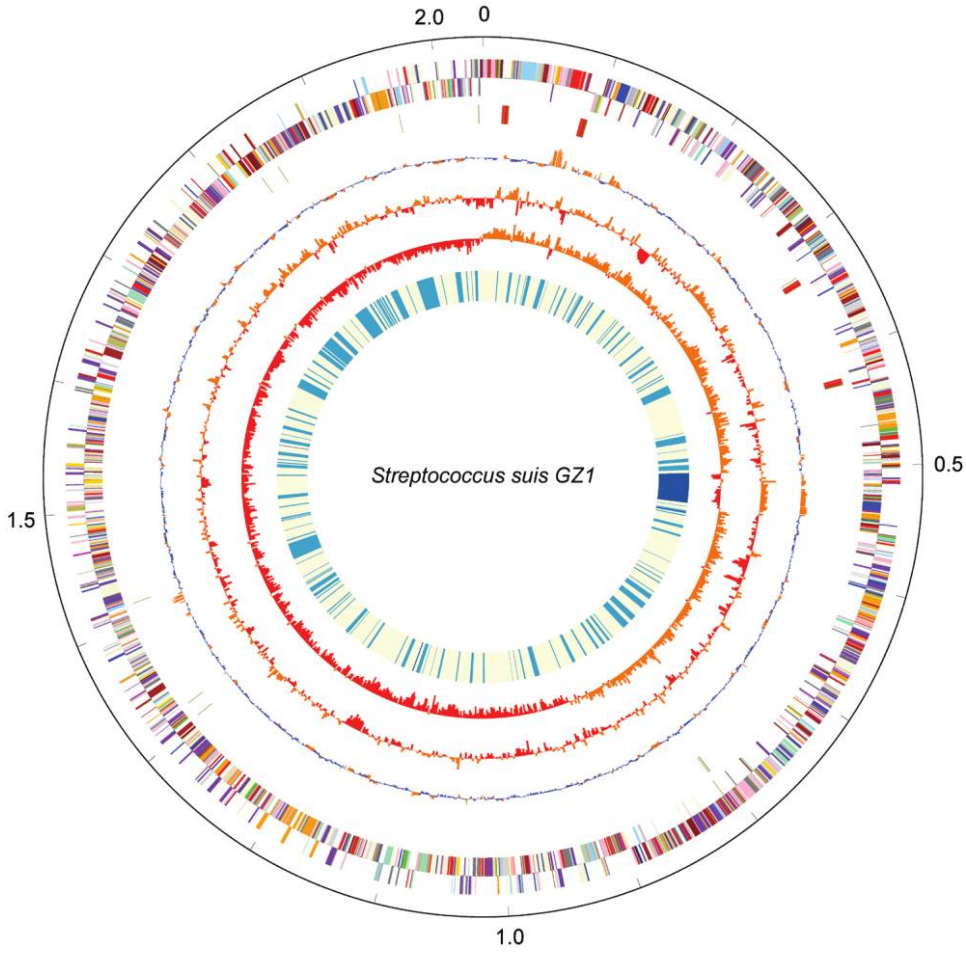

Figure 2. Circular representation of the genome of highly pathogenic strain ST1 GZ1 of *Streptococcus suis*. The circles (from outside to inside) represent features: The first, or outermost, circle shows the scale (in megabases). The second circle comprises paired rings showing the predicted coding sequences on the plus strands (outer ring) and minus strands (inner ring). All genes are color coded by function and are classified on the basis of clusters of orthologous groups of protein: *aquamarine*, energy production and conversion; *ivory*, carbohydrate transport and metabolism; *navy*, cytoskeleton; *blue*, transcription; *blue-violet*, amino acid transport and metabolism; *brown*, defense mechanisms; *dark khaki*, lipid metabolism; *dark red*, posttranslational modification, protein turnover, and chaperones; *dark salmon*, RNA processing and modification; *gold*, coenzyme metabolism; *gray*, function unknown; *green*, cell division and chromosome partitioning; *light cyan*, intracellular trafficking, secretion, and vesicular transport; *light gray*, general function prediction only; *light sky blue*, nucleotide transport and metabolism; *beige*, chromatin structure and dynamics; *magenta*, secondary-metabolite biosynthesis, transport, and catabolism; *light steel blue*, cell motility and secretion; *orange*, cell-envelope biogenesis and outer membrane; *pink*, DNA replication, recombination, and repair; *purple*, inorganic-ion transport and metabolism; *red*, translation, ribosomal structure, and biogenesis; and *wheat*, signal transduction. The third circle denotes rRNA *(red)* and tRNA *(olive)*. The fourth circle shows the dinucleotide bias of genes, with the obvious ones shown in *red*. The fifth circle shows the G+C content, or G+C skew. The sixth, or innermost, circle represents the acquired islands found in the unfinished sequnce of strain ST7, compared with the sequence of strain ST25 89/1591.

cytopenia, and abnormal coagulation profiles, which are characteristic of purpura fulminans (table 1).

This is the first study to analyze cytokines during the acute phase of *S. suis* infection in humans. Serum levels of TNF-α, IL-1β, IL-6, IL-8, IL-12p70, and IFN-γ were significantly higher in 6 patients with STSLS than in 9 patients with meningitis only (table 1) [25]. This finding strongly suggests the involvement of a mechanism that triggers a proinflammatory cytokine cascade, leading to rapidly progressive systemic inflammatory responses, which may contribute to both the sudden onset of the disease and the high mortality among patients with STSLS.

To better understand the relationship between pathogen genotype and elevated serum levels of cytokines associated with STSLS, we used a mouse-infection model to compare cytokine production induced by 3 representative *S. suis* genotypes—intermediately pathogenic strain ST25 89/1591, highly pathogenic strain ST1 GZ1, and epidemic strain ST7 SC84. Our results indicate that ST7 SC84 induces significantly higher serum levels of TNF-α, IL-1β, IL-6, IL-12p70, KC, and MCP-1 than does either ST1 GZ1 or strain ST25 89/1591. Levels of cytokines TNF-α, IL-1β, IL-6, IL-12p70, KC, and MCP-1 were also significantly higher in ST1-infected mice than in ST25-infected mice. These

Table 2. Genomic islands acquired or lost by *Streptococcus suis* sequence type 1 (ST1) (GZ1) and sequence type 7 (ST7) (05ZYH33).

	Genomic islands, no.						
	Acquired				Lost		
Category	ST1	ST7	ST1+ST7	ST7 specific	ST1	ST7	ST1+ST7
Antibiotic resistance	1[a]	1[b]		1[b]			
Cell structure	5	5	5		2	2	2
DNA recombination	6	5	4	1	11	12	9
DNA/RNA processing	11	10	10		8	8	8
Unknown function	21	21	21		18	14	14
Immunogenic protein	1[c]	1[c]	1[c]				
Metabolism	43	43	43		13	9	9
Other	4	4	4				
Phage related	1	1	1		5	5	5
Regulation	9	9	9		3	1	1
Signal transduction	6	8	6	2			
Transport	22	23	21	2	3	3	3
Virulence related	5	5	5				
Total	132 (135)[d]	133 (136)[e]	128 (130)[f]	5 (6)[g]	63	54	51
Total size, bp	478,262	496,026	429,197	66,829	338,053	300,716	296,846

[a] Strain ST1 GZ1 has an antibiotic-resistance island including the tetracycline-resistance gene *tetW*.
[b] Strain ST7 has an antibiotic-resistance island including the tetracycline-resistance gene *tetM*.
[c] Specific antibody to acquired island 9 (GroEL) was detected in convalescent sera from infected patients.
[d] Acquired islands 87, 96, and 134 were proposed to have virulence-related and phage-related functions, virulence-related and signal-transduction functions, and signal-transduction and antibiotic-resistance functions, respectively.
[e] Acquired islands 33, 87, and 96 were proposed to have antibiotic-resistance and DNA/RNA-processing functions, virulence-related and phage-related functions, and virulence-related and signal-transduction functions, respectively.
[f] Acquired islands 87 and 96 were proposed to have 2 functions.
[g] Acquired island 134 was proposed to have signal-transduction and antibiotic-resistance functions.

results provide the first evidence that strains ST7 and ST1 are able to stimulate the host immune system to produce extremely high levels of proinflammatory cytokines, which might have been responsible for the shock syndrome observed in *S. suis* infections in humans. Interestingly, the mouse-infection model has recently been used to evaluate cytokine expression induced by an ST1 strain, and the results are similar to those of the present study [26]. The cytokines analyzed in both studies are known to play an important role in septic shock. High levels of TNF-α and IL-6 correlate inversely with survival time in patients with sepsis and also play an important role in shock [27]. Similarly, IFN-γ contributes to immune control of invading pathogens but also, when its production is excessive or uncontrolled, may cause pathology leading to death [28–30]. In the present study, IFN-γ was found to be significantly elevated in human patients with STSLS but not in mice infected with *S. suis*, indicating species-specific differences. Nevertheless, other studies have suggested that this important Th1 cytokine contributes to toxic shock in humans [31]. Other cytokines, such as IL-1 and IL-12, also play an important role in sepsis [32, 33]. Moreover, chemokines are key factors in acute inflammation, as has recently been reported with regard to *S. suis* [26]. In the present study, we have shown that intermediately and highly pathogenic strains of *S. suis* can induce distinct chemokine profiles.

In addition to the proinflammatory cytokine cascade, we also have shown for the first time that epidemic strain ST7 induces the highest level of toxicity to PBMCs, followed by highly pathogenic strain ST1 GZ1 and, finally, intermediately pathogenic strain ST25 89/1591. ST25 is not known to produce suilysin, which may explain, at least in part, its lower cytotoxicity. Whether the greater cytotoxicity of ST1 GZ1 is related to its higher production of suilysin or to other virulence factors is unknown; ongoing experiments in our laboratory are exploring these possibilities.

To elucidate the genetic events underlying the emergence and increased virulence of highly pathogenic strain ST1 and epidemic strain ST7, we sequenced the genome of ST1 GZ1, which was isolated at approximately the same time that ST7 was isolated during the outbreak in Sichuan Province, from a patient in Guizhou Province who had septicemia (figure 2) [16]. ST1 GZ1 was selected for comparison because it was considered to be an appropriate reference strain to reveal genetic changes in ST7, which thus far has been isolated only in China. It should be mentioned that all isolates from patients in the present study who were infected with culture-confirmed *S. suis* showed identical *Sma*I restriction patterns, as demonstrated by pulse-field gel electrophoresis.

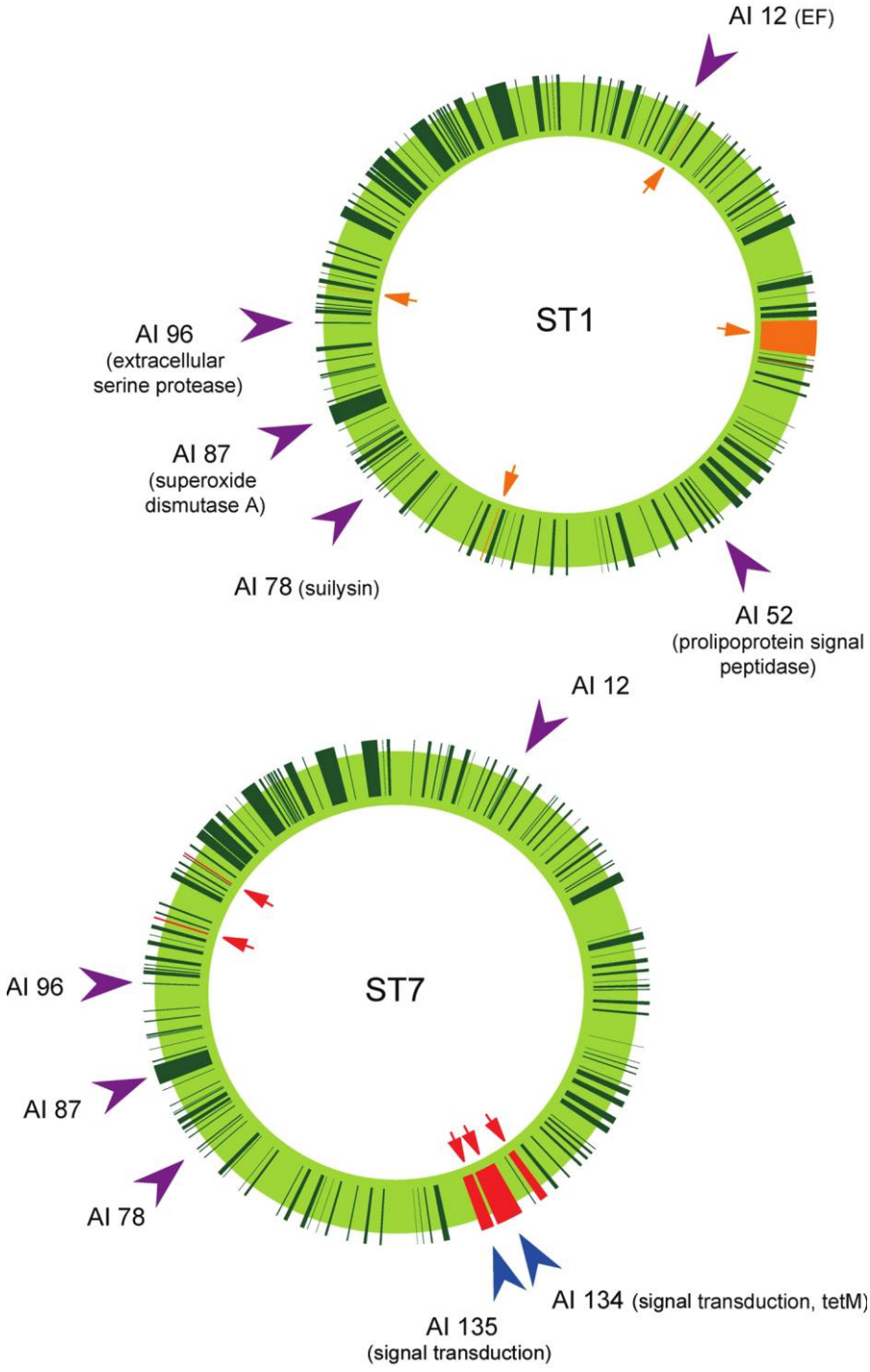

Figure 3. Genomic islands acquired (AI) by highly pathogenic strain ST1 *(upper circle)* and epidemic strain ST7 *(lower circle)* of *Streptococcus suis*. On the basis of comparison with the unfinished genome sequences of intermediately pathogenic strain ST25 89/1591, ST1 GZ1 was found to have acquired 132 genomic islands *(darker green)*, including 5 pathogenicity islands; of these 132 acquired islands, 4 *(orange)* were ST1 specific. Compared with the unfinished genome sequences of ST25 89/1591, ST7 05ZYH33 was found to have acquired 133 genomic islands *(darker green)*. Of these 133 acquired islands, 5 *(red)* were ST7 specific. The acquired islands include genes that encode recognized virulence factors, such as suilysin and extracellular factor (EF), as well as 2 signal-transduction genomic islands *(indicated by arrows)*. There are 5 ST7-specific AIs *(red arrows in lower circle)*: (1) an AI that encodes a recombinase; (2) an AI that encodes determinants involved in tetracycline resistance and in the NisK-NisR–like 2-component signal-transduction system; (3) an AI that encodes factors involved in the SalK-SalR-like 2-component signal-transduction system; (4) an AI that encodes an adenosine triphosphate–binding cassette (ABC)–type metal-ion transporter; and (5) an AI that encodes a peptide ABC transporter. The NisK-NisR 2–like system regulates the biosynthesis and immunity of nisin, whereas the SalK-SalR–like system regulates the biosynthesis and immunity of salivaricin.

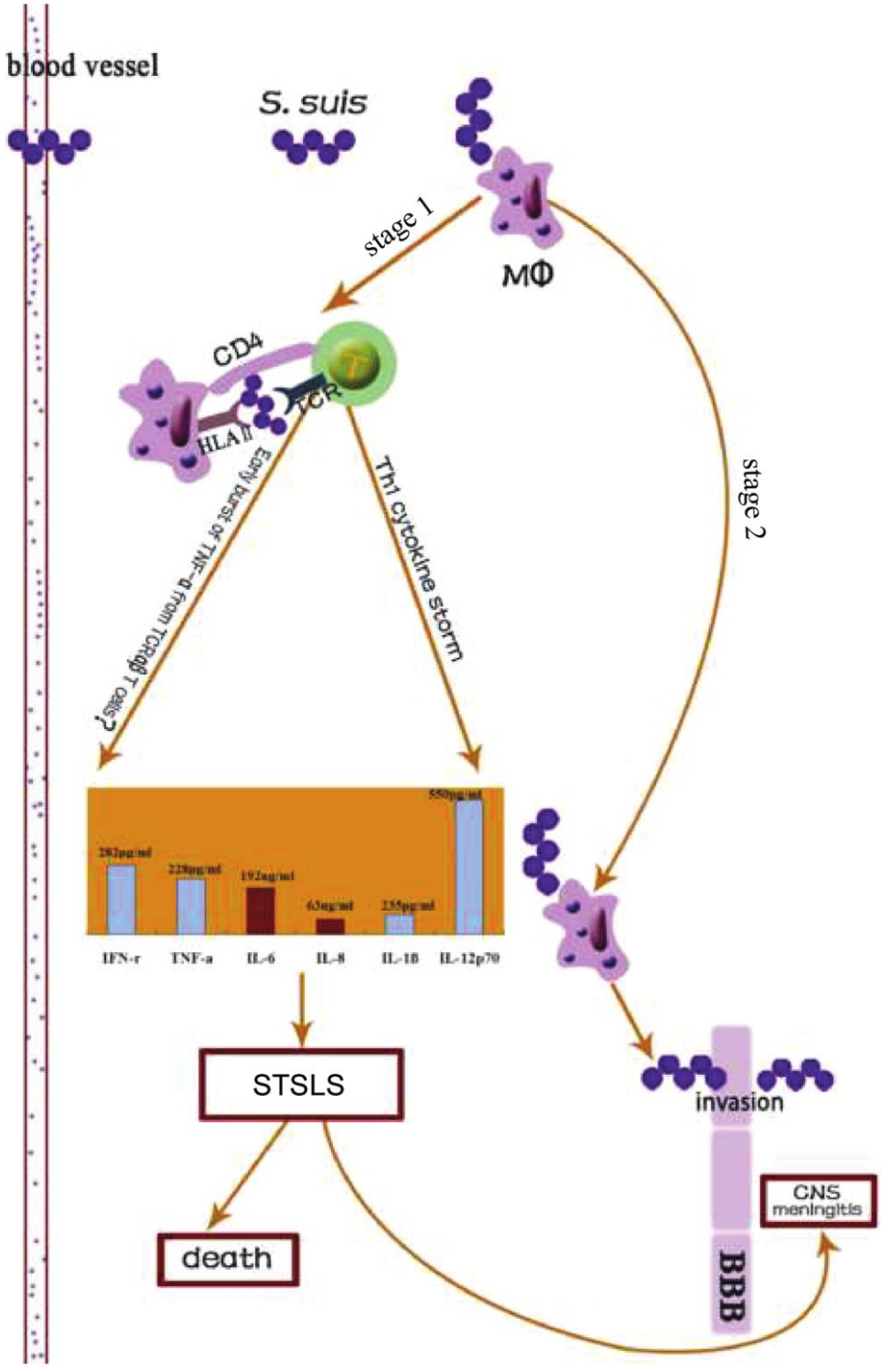

Figure 4. Proposed 2-stage model of pathogenesis of infection with strain ST7 of *Streptococcus suis*. In stage 1, interactions between invading *S. suis* and the host immune system result, via unknown mechanisms, in an early burst of proinflammatory cytokines, including Th1 cytokines, tumor-necrosis factor (TNF)–α and interleukin (IL)–1β. These inflammatory responses may lead to shock and sudden death as early as 13 h after infection. The inset *(bar graph)* shows the serum levels of cytokines in patients presenting with streptococcal toxic-shock-like syndrome (STSLS). In stage 2, which develops over several days, the pathogen uses virulence factors such as suilysin to cause disease—specifically, meningitis. BBB, blood-brain barrier; CNS, central nervous system; IFN, interferon.

Comparative analysis of *S. suis* genomes has revealed that highly pathogenic strain ST1 GZ1 acquired 132 AIs, including 5 pathogenicity islands, when it evolved from intermediately pathogenic strain ST25, and that epidemic strain ST7 acquired another 5 AIs with 64 CDSs comprising 66,829 bp when it evolved from ST1 [2, 19, 20] (table 2 and figure 3; also see table A2 in the appendix, which is available only in the electronic edition of the *Journal*). The potential significance of the acquisition of these genes requires further investigation. However, on the basis of the results of the present study, we suggest that the genomic islands acquired by the highly pathogenic and epidemic strains of *S. suis* are closely associated with the unique clinical presentation and epidemiology of the Sichuan outbreak, the elevated cytokine levels in patients with STSLS and in experimentally infected mice, and the enhanced cytotoxicity to PBMCs. Additional studies evaluating the role that these genomic islands play in the pathogenesis of *S. suis* infection are warranted.

On the basis of the results of the present study and other published data, we propose the following 2-stage model of the pathogenesis of *S. suis* strain ST7 (figure 4). In stage 1, on entry of the pathogen into the bloodstream, bacterial cell-wall components interact with the host immune system via pattern-recognition receptors such as Toll-like receptor 2 and CD14 [34, 35] and probably the 2-component signal-transduction system. These host-pathogen interactions trigger a burst of proinflammatory cytokines, which may contribute to the development of toxic shock. In the present study, we have shown that ST7 has evolved to acquire the ability to stimulate the host immune system to produce massive amounts of proinflammatory cytokines such as TNF-α, IL-1 and IFN-γ, which, in turn, can induce high levels of other cytokines, such as IL-6 and IL-12, leading to STSLS (figure 1). If the patient survives stage 1, then, in stage 2, the pathogen, via the bloodstream, can travel freely or bind to monocytes to reach the central nervous system and cause meningitis [36]; this is the typical clinical presentation of infections caused by either strain ST1 or strain ST25. Simple antibiotic therapy, once thought to be effective [24, 37], continues to be widely used to treat *S. suis* infection in humans [38–41]. However, the results of the present study indicate that, to prevent sudden death associated with ST7 or ST1 *S. suis* infections, treatment should be initiated early during the acute phase of infection and should include therapies to prevent or reduce the risk of STSLS.

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